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Analytic comparison of talc in commercially available baby powder and in pelvic tissues resected from ovarian carcinoma patients

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HIGHLIGHTS

- 77.7%– of particles in talcum powder have aspect ratio of 1–3.9 and area of 1–400 μm^2.
- 83.5% of talc particles in pelvic tissues ovarian carcinoma patients have an aspect ratio of 1–3.9 and an area of 1–400 μm^2.
- These similarities are statistically significant.

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ABSTRACT

Objective. Measure the size and shape of talc particles in talcum powder and compare this data to the size and shape of talc particles found in surgically resected tissues from patients with ovarian carcinoma.

Methods. Using polarized light microscopy (PLM) and scanning electron microscopy (SEM), we measured the size and shape of talc particles in samples of talc-containing baby powder (TCBP) and surgically resected pelvic tissues (hysterectomies) from talc-exposed patients with ovarian carcinoma.

Results. The most frequent class of particles in TCBP can be unequivocally identified as talc, using both polarized light microscopy and scanning electron microscopy with energy dispersive X-ray analysis (SEM/EDX). The talc particles found in resected tissues from ovarian carcinoma patients are similar in size and shape to the most abundant morphological class of particles in TCBP.

Conclusions. This finding, combined with previous epidemiological literature and tissue-based analytical studies, provides further evidence that the small, isodiametric particles that dominate TCBP can migrate from the perineum and become lodged in distal structures in the female reproductive tract, where they may lead to an increased risk of developing ovarian carcinoma.

1. Introduction

The chemical formula for the mineral talc is $Mg_3Si_4O_{10}(OH)_2$. Cosmetic talc (CAS No. 14807–96-6) is a powder of native hydrous magnesium silicate, with small amounts of contaminating aluminum silicate [1]. Talc-containing baby powder (TCBP) is produced by mining talc, grinding, sifting, and adding fragrances [2,3]. TCBP has been widely used for many years both for infant skin care and for personal hygiene, e.g., perineal application. In recent decades there has been strong suspicion that the long-term use of such products may increase the likelihood of developing ovarian cancer, specifically epithelial ovarian carcinoma. Many epidemiological studies indicate that there is a clear excess, often statistically significant, in the incidence of ovarian cancer with the long-term use of commercially available talc powder, with a risk ratio being about 1.3 [4–8]. Recent studies have explained the association of talc with ovarian carcinoma in molecular terms, specifically the generation of a proinflammatory and proöxidant state that renders mutations and increased cell proliferation more likely [9].

Talc and other materials can migrate from the perineum to the ovary and/or other pelvic tissues via the transport system in the female reproductive tract that normally moves ova proximally and spermatozoa distally. Vandemark and Moeller [10] showed that in estrous cows, spermatozoa reached the oviducts within 2.5 min of mating, far sooner than would be expected if spermatozoan motility were solely

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responsible for this rapid transport, suggesting that smooth muscle contraction is an important driver of distal transport of spermatozoa. Oxytocin, a hormone released during copulation in cows and humans, stimulates smooth muscle contraction in the female reproductive tract [11], thus accelerating distal transport of spermatozoa as well as non-motile particles from the vagina to the uterine tubes and ovary. Egli and Newton [12] inserted carbon particles in the vagina of two healthy women before hysterectomy, recovering them in the uterine tubes about 30 min later. Radiographic tracer studies show that labeled particles can migrate from the external genitalia to internal pelvic structures via lymphatics [13–15]. Thus, it is reasonable to assume that talc particles in TCBP may migrate from the perineum to the uterine tubes and ovary in substantial numbers. It is also reasonable to assume that there may be differences in patients regarding the transport ability and speed, and that migration pathways for talc or other exogenous substances may be idiosyncratic. The purpose of the study reported here was to determine the range of particle sizes in TCBP in comparison to the size of particles found in pelvic tissues of ovarian cancer patients with a history of perineal talc use. Particle size has not been a focus of previous studies identifying talc particles in pelvic tissues.

Ovarian cancers are heterogeneous, but as a general category, they are the most lethal gynecologic malignancy [16]. Although many ovarian cancer cases have an uncertain etiology, BRCA mutations are involved in some cases [17–19]. The epidemiological association of ovarian carcinoma, i.e., cancers with epithelial cell origin, with talc use has been previously mentioned [4–8]. In contrast, oral contraceptives, multiple pregnancies, and tubal ligation appear to reduce the incidence of ovarian carcinoma [20]. In this study, we showed that the most frequent class of small, roughly isodiametric particles found in commercial talcum powder samples are very similar to the most frequent class of small, roughly isodiametric talc particles found in surgically resected tissues from ovarian carcinoma patients.

2. Materials and methods

2.1. Sample description

TCBP samples were purchased at drug stores; or, from Amazon.com; or, partially used samples were obtained from personal contacts. The samples used in this study are described in **Supplementary Material** Table 1.

2.2. Microscopy

Bright field, differential interference contrast (DIC), polarized light microscopy (PLM), and scanning electron microscopy (SEM) with energy dispersive X-ray analysis (SEM/EDX) were used to study both TCBP samples and surgically resected tissues from TCBP-exposed human patients with ovarian cancer. For each sample of TCBP, a small amount was placed on a standard glass slide and covered with a drop of Permount and a coverslip; or, on a SEM stub with adhesive. For LM, we used a Zeiss AXIO Imager A.2 light microscope equipped with a Zeiss Axiocam MRm camera, with Zeiss Axiovision software. For SEM/EDX we used a field emission SEM low vacuum instrument (FEI Teneo) with 50 pA beam current, 10 or 20 kV beam acceleration, 10 mm working distance, and condenser lens current of 400 pA. Spectra were generated by an EDAX AMETEK system using EDAX software and vertical counts collected for 200 s. An individual particle was identified as talc if it showed only oxygen, magnesium, and silicon major peaks with the Mg/Si atomic weight percent ratio within the theoretical value of 0.649 ± 5%. We measured the width and length of randomly chosen particles from different TCBP samples or from resected tissues in the SEM. The length (L) was the longest dimension of the particle and the width (W) was the longest dimension perpendicular to the length. The aspect ratio (AR) was then calculated as L/W. The area of the particle was simply LXW.

Table 1
Aspect ratio and total area of 400 randomly selected TCBP particles from SEM and 200 talc particles from ovarian carcinoma resections.

Aspect Ratio, Area μm^2	Number	%Total
400 Sample TCBP		
1–3.9, 1–400	311	77.7
1–2, > 400	56	14.0
4.0- > 10, 1–200	33	8.3
200 Ovarian Carcinoma Talc		
1–3.9, 1–400	167	83.5
1–2, >400	0	0.0
4.0- > 10, 1–200	33	16.3

2.3. Resected tissues from ovarian carcinoma patients

We also conducted a detailed study of talc particles from 11 different, randomly selected patients with epithelial ovarian carcinoma (Table 2). As shown in Table 2, the ages of the 11 patients ranged from 36 to 69, and FIGO stages from I to IV, and nine patients had serous carcinoma, one endometrioid carcinoma, and one mixed serous-endometrioid carcinoma. We measured and recorded the dimensions of 200 talc particles as described above. All 11 patient cases were received for consultative purposes by JJG, each representing a patient with ovarian carcinoma and a significant known history of perineal talc use. Surgical pathology reports were provided for each patient. All patient identifiers, including the 18 recognized HIPPA identifiers (https://www.atlanta.va.gov/Docs/HIPPA Identifiers.pdf), were removed prior to final assembly of the data and submission for publication. Hematoxylin and eosin (H&E)-stained sections from resections were reviewed. All slides were analyzed with an Olympus BH-2 LM with dual, rotating polarizing filters. Each slide was scanned systematically and completely at 200× to identify birefringent particles in the same plane of focus as the tissue. Birefringent particles located outside the contours of the tissue, or within the tissue, but less than the width of one high-power (400×) field away from the tissue surface were assumed to be laboratory contaminants and thus not included in the assessment [21].

Paraffin blocks, corresponding to histological slides having the most abundant birefringent intracellular particles in the tissue were used for in situ SEM/EDX as described by Thakral and Abraham [22]. As a precaution against contamination, the blocks were handled with particle-free gloves on precleaned surfaces and were sectioned, thus removing ~30 μm of tissue and paraffin using a rotary microtome with a fresh, cleaned, blade. This removed surface contamination from previous handling. The blocks were always kept in closed containers to prevent any laboratory contamination. The tissue surfaces were studied with a Hitachi SU6600 field emission SEM with an Oxford EDX with Aztec version 2.0 to 3.3 software, and EDX detector model X-Max 50 SDD. The backscatter mode of the microscope highlighted mineral particles within the tissues. Particles were examined to assess morphological characteristics and to carry out spectral analysis with EDX on each particle found. Talc particles were characterized by magnesium (Mg) and silicon (Si) peaks falling within ±5% of the theoretical atomic ratio of 0.75 and atomic weight percentage ratio of 0.649 [21].

3. Results

3.1. Size distribution of randomly chosen talc particles

We gathered details on the morphology of particles in various TCBP samples so that we could subsequently compare them with talc particle morphology in surgically resected specimens from patients with

Table 2
Size Distribution of 200 Talc Particles in Surgically Resected Pelvic Tissues from 11 Patients with Ovarian Carcinoma.

Case Number	Patient age	Carcinoma Type	Anatomic stage	Resected Tissue Site(s)	Number of Particles Analyzed	Average Area ± s.d. μm^2	Average Aspect Ration ± s.d.
1	49	serous carcinoma, poorly differentiated	T3b N1 MX (FIGO Stage III)	**Omentum**	**3**	**64.1 ± 49.3**	**1.4 ± 0.4**
2	37	endometrioid carcinoma, well-differentiated	T1a N0 MX (FIGO Stage I)	**ovarian fossa and r. pelvic lymph nodes**	**6**	**31.8 ± 21.2**	**2.0 ± 0.7**
3	55	serous carcinoma, moderately differentiated	T2b N0 MX (FIGO Stage II)	**r. ovary and cervix**	**54**	**20.1 ± 33.1**	**3.0 ± 11.7**
4	57	mixed serous and endometrioid carcinoma, well differentiated	T1a N0 MX (FIGO Stage I)	**r. iliac lymph nodes**	**16**	**34.0 ± 20.4**	**1.6 ± 0.4**
5	59	serous carcinoma, high grade	T3c N1 MX (FIGO Stage III)	**l. pelvic lymph nodes**	**11**	**56.2 ± 56.0**	**2.5 ± 1.4**
6	48	Serous carcinoma poorly differentiated	T3b N1 MX (FIGO Stage III)	**l. and r. ovary**	**5**	**24.3 ± 14.8**	**3.3 ± 0.7**
7	38	serous carcinoma, (moderately differentiated)	T3c NX M1 (FIGO Stage IV)	**l. tube and ovary**	**7**	**17.3 ± 10.5**	**2.6 ± 0.9**
8	52	serous carcinoma, high grade	T3c NX MX (FIGO Stage III)	**r. ovary**	**6**	**13.0 ± 16.6**	**2.7 ± 1.8**
9	48	serous carcinoma, high grade	T3c NX MX (FIGO Stage III)	**Omentum**	**8**	**36.6 ± 26.8**	**1.5 ± 0.4**
10	36	serous carcinoma, well differentiated	T3a NX MX (FIGO Stage III)	**r. pelvic and** l. **para-aortic lymph nodes**	**16**	**36.0 ± 29.4**	**1.5 ± 0.5**
11	69	serous carcinoma, high grade	pT3c N1 MX (FIGO stage III)	**r. ovary and pelvic lymph nodes, l. ovary**	**68**	**14.0 ± 35.6**	**2.9 ± 1.6**

ovarian carcinoma. TCBP samples had a variety of dimensions of particles but were all essentially alike in both the PLM and SEM. Talc particles were birefringent and consisted of a mixture of large and small particles (range 1–65 μm diameter) (Fig. 1).

Most particles were either small or large and approximately isodiametric with aspect ratios in the 1–2 range. Detailed analysis of 400 randomly chosen TCBP particles with SEM revealed three main size classes: the most common class, (aspect ratio 1–3.9 and small area 1–400 μm^2; 77.7%); and, two other uncommon classes; one with a small aspect ratio (1–2) and large area (> 400 μm^2; 14.0%); and, the other with a large aspect ratio (4- > 10) but a small area (1–200 μm^2, 8.3%). These results are summarized in Table 1.

These measurements were essentially consistent and reproducible. For example, of 600 particles from different samples of Johnson &

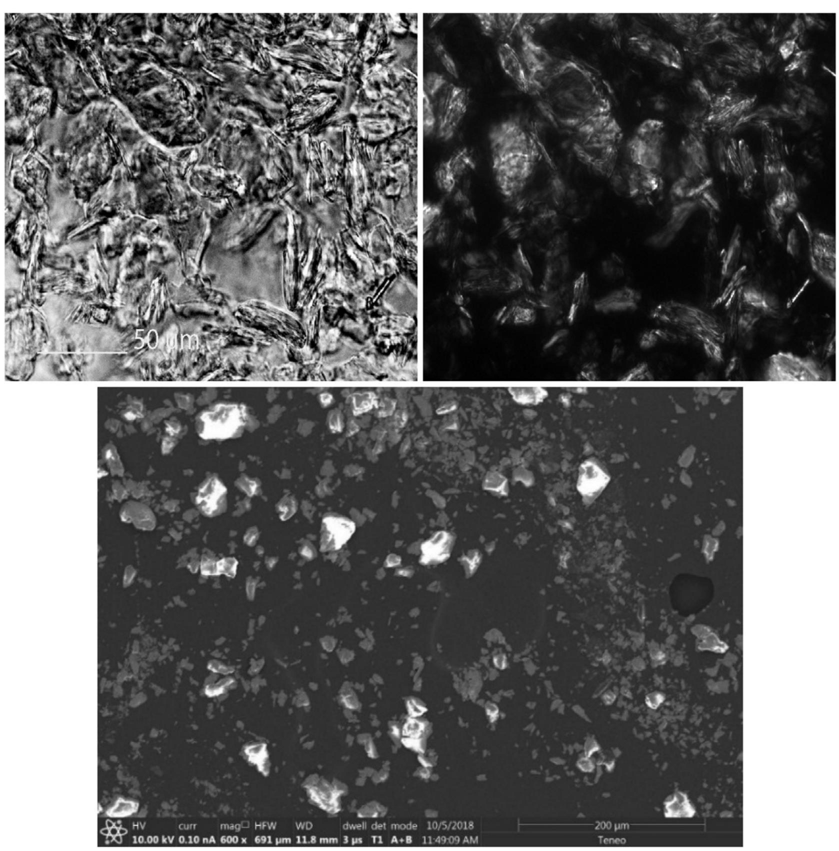

Fig. 1. Differential interference contrast microscopy (top left), polarized light microscopy (top right) of the same field of a typical sample of talc-containing baby powder. Scale bar on left = 50 μm and applies to both images. Scanning electron micrograph of a typical sample of talc-containing baby powder (bottom). Scale bar = 200 μm.

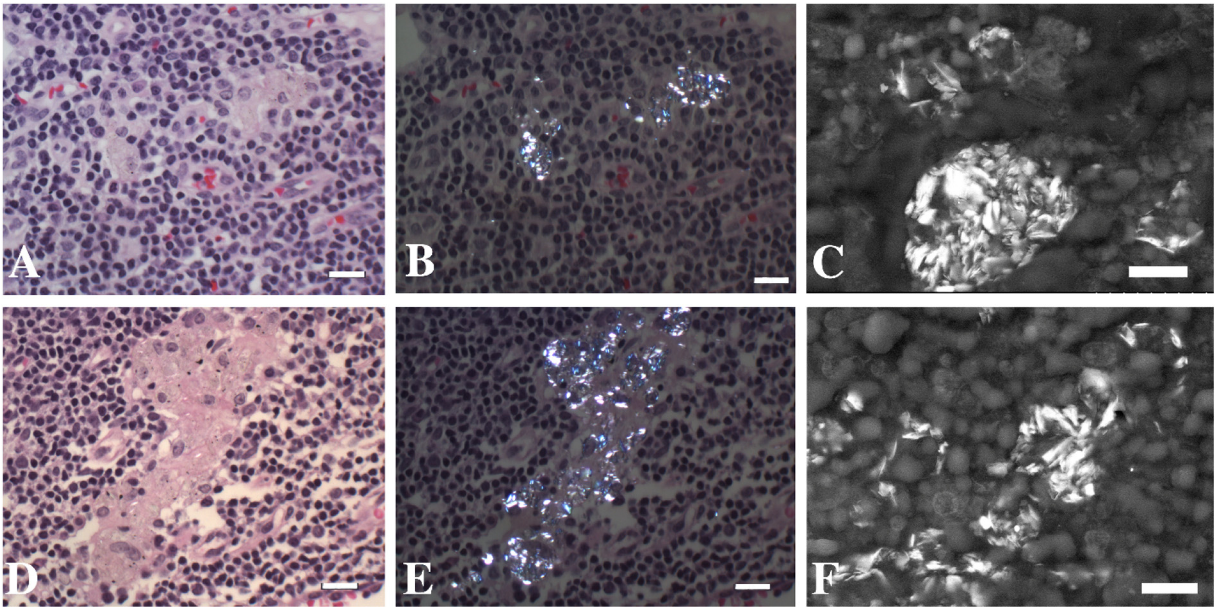

Fig. 2. A,B,D, and E are H&*E*-stained lymph nodes from Patient 11. The light micrographs in A and D showed two nondescript patterns in macrophages in pelvic lymph nodes. Polarized light microscopy of these exact same fields (B and E) showed these macrophages filled with small birefringent particles. Scanning electron micrographs on the tissue block from which the slide was made (but of course at a different level in the tissue from the histology slide) showed similar aggregates of particles in macrophages and all of the particles were less than 4 μm in greatest dimension, and many are less than 1 μm. Light microscopy bars are 20 μm. SEM bars are 4 μm.

Johnson and Caswell-Massey talcum powders, 81.0% were 1–3.9 AR, 1–400 μm^2 area, 15.8% were 1–2 AR, > 400 μm^2, and 3.2% were 4.0 > 10 AR, 1–200 μm^2 area.

3.2. Identification of particles in talc samples with SEM/EDX

Under SEM analysis, the TCBP samples showed a wide variety of particles, most small and roughly isodiametric, some large and roughly isodiametric, and a few elongated fibers with a high aspect ratio (Fig. 1). With SEM /EDX, both small and large isodiametric and fibers show oxygen, magnesium, and silicon spectral signals characteristic of talc in the appropriate Mg/Si ratio (**See Supplemental Material,** Figs. 1 and 2). We examined 29 particles from three different samples of TCBP by SEM/EDX. A large (66 × 58 μm, AR = 1.1) particle and a small particle (2.1 × 1.3 μm, AR = 1.6) both showed typical talc signals. Of the remaining 27 particles, with a range of AR 2.4 to 13.0, average = 6.0 ± 2.7 s.d., 24 produced typical talc spectra, but two gave talc with a trace of aluminum and one gave talc with a trace of aluminum and iron.

3.3. Analysis of talc particles in ovarian carcinoma resections

It was essential to gather morphological measurements on talc particles found in surgically resected tissues from ovarian carcinoma patients. All ovarian carcinoma patients had a substantial, long-term exposure to either Baby Powder or Shower to Shower talc-containing products manufactured by Johnson & Johnson. In surgically resected tissues from talc-exposed patients with ovarian carcinoma, a subset of tissues studied with PLM revealed birefringent particles commonly in lymph nodes, ovaries, and other pelvic tissues. The particles were most often within benign tissue, reactive fibroblastic tissue, or chronically inflamed tissue near tumor, but not typically within tumor itself. In lymph nodes, birefringent particles typically were clustered within macrophages (Fig. 2A, B, D, E).

When paraffin-embedded blocks of different areas of these same tissues were examined with SEM/EDX, many particles gave talc spectral signals with peaks for oxygen, magnesium, and silicon with Mg/Si ratios indicative of talc (Figs. 2C, F, Fig. 3, Fig. 4**)**. We collected measurements on 200 talc particles in surgically resected tissues from 11 ovarian carcinoma patients (Table 2). The range of dimensions was from the

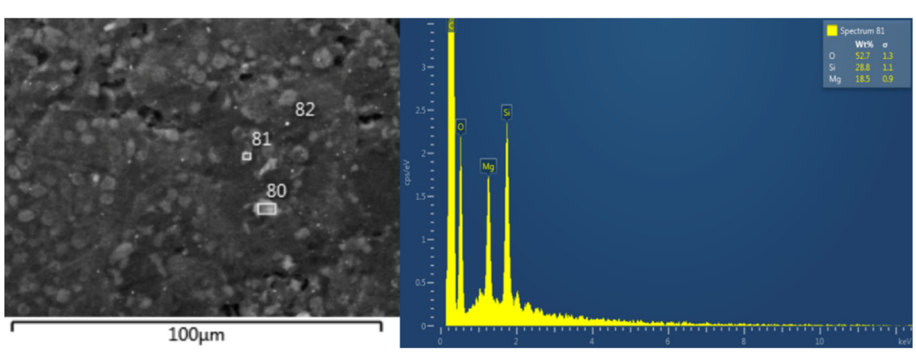

Fig. 3. Left, SEM image of three small particles in a left iliac lymph node from patient 4. Right, EDX spectrum of particle 81 with peaks for magnesium, silicon, and oxygen. All three particles are magnesium silicates, but only particle 81 is within the ±5% of the theoretical atomic weight % of talc (0.649) at 0.642. The particle is 1.5 μm in greatest dimension.

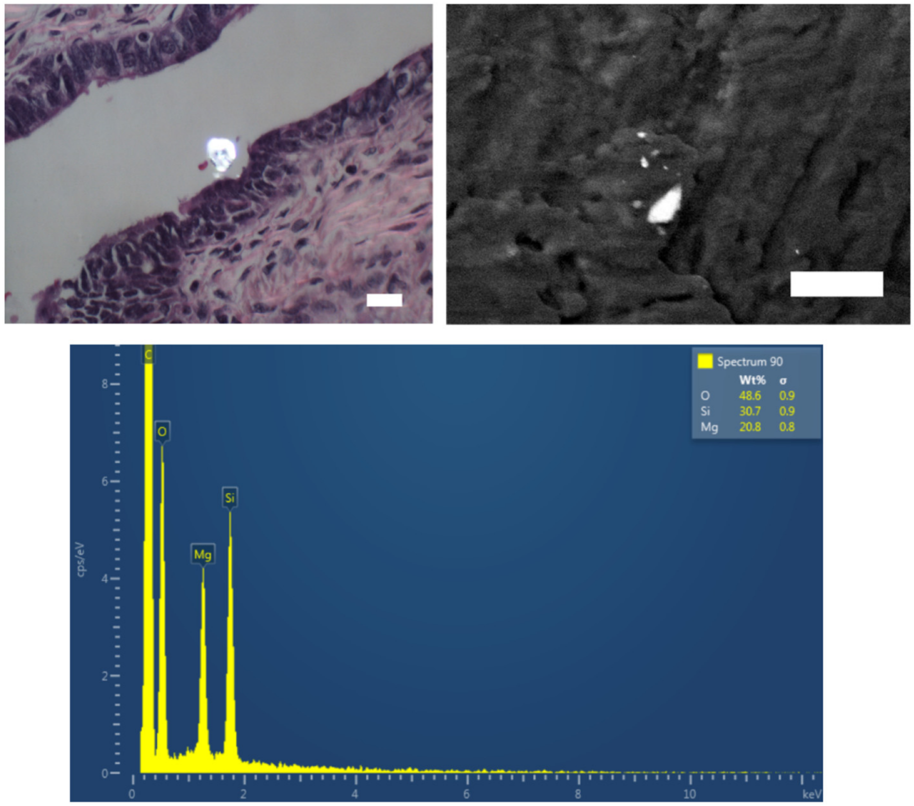

Fig. 4. Top Left, PLM showing large and small particles on the luminal epithelial surface of the uterine tube in Patient 11. Bar = 20 μm. Top Right, SEM image of particles along the luminal epithelial surface of the uterine tube also showed small and large particles. Bar = 20 μm. Below, EDX spectrum from a small particle on the luminal epithelial surface of the uterine tube with a Mg/Si atomic weight ratio of 0.677 within ±5% of the 0.649 theoretical Mg/Si ratio for talc.

smallest particle, which was 0.9 μm long and 0.8 μm wide (area 0.72 μm^2, AR 1.1); to the largest particle 28.9 μm long and 6.2 μm wide (area 179.18 μm^2, AR 4.7). These results show that small, roughly isodiametric particles of talc, when applied externally, can make their way through the female reproductive tract and become embedded in tissues of surgical resections from ovarian carcinoma patients. In addition, we found that 185/200 (92.5%) of the talc particles in association with malignant ovarian tissue were small, with length in the 0.9–10 μm range, interestingly, quite similar to the most frequent class of particles in control TCBP. All measurements for these 200 particles from 11 ovarian carcinoma patients are shown in Table 2**.** We also summarized the data from talc particles in ovarian carcinoma resections in Table 1**,** using the same size groupings we used for TCBP**.** A scatter plot of 200 TCBP particles and all 200 talc particles in ovarian carcinoma resections, plotting aspect ratio as a function of particle area, is shown in **Supplemental Materials** Figs. 3 and 4**.**

The range of particle sizes, based on the maximum length for all 200 talc particles was from 0.9 μm to 28.9 μm (average = 5.78 μm ± 4.02 s.d.). The range of aspect ratios was from 1 to 11.17 (average = 2.49 ± 1.51 s.d.). These particles fell into two classes: the most common class, (aspect ratio 1–3.9 and small area 1–400 μm^2; 83.5%); and, the other with a large aspect ratio (4- > 10) but a small area (1–200 μm^2; 16.3%). We found no particles with a small aspect ratio (1–2) and large area (700–4425 μm^2; 0.00%). All 200 particles described in Table 2 are talc based on SEM/EDX spectral analysis. A typical example of SEM/EDX analysis of these particles is shown in Fig. 3**.** These results are summarized in Table 2 **and Supplementary Materials Figs. 5 and 6**.

It is important to note that the vast majority of the particles of talc found in resections from ovarian carcinoma patients had both a small area and small aspect ratio. We found elongated fibers of talc with a large aspect ratio in these resected specimens rarely.

In resections from ovarian carcinoma patients, uterine tubes are typically included as part of the hysterectomy. In these more proximal locations in the female reproductive tract, a small number of birefringent particles, some identified as talc by SEM/EDX, assumed to be talc originally applied to the perineum, were also present (Fig. 4).

In patient 3, a case of serous ovarian carcinoma, we found 53 talc particles in the cervix. In this subset of particles, the size statistics (mean ± standard deviation) were as follows:

1) Average Length = 6.30 μm ± 5.02 s.d range 0.90–28.90 μm
2) Average Width = 2.33 μm ± 1.57 s.d. range 0.54–7.90 μm
3) Average Area = 20.10 μm^2 ± 33.09 s.d. range 0.68–179.18 μm^2
4) Average Aspect ratio = 2.98 ± 1.75 s.d. range 1–11.17

These do not appear to be significantly different in dimensional statistics from the total group of talc particles in all other specimens (147 total particles):

1) Average Length = 5.51 μm ± 3.76 s.d range 1.06–17.10 μm
2) Average Width = 3.17 μm ± 2.57 s.d. range 0.15–14.3 μm
3) Average Area = 25.31 μm2 ± 35.05 s.d. range 0.41–244.53 μm^2
4) Average Aspect ratio = 2.32 ± 1.38 s.d. range 1–6.93

These cervical talc particles likely originate from perineal application of talc. They may have reached the cervical stroma through erosions in the overlying epithelial surface, and/or have been transported to deeper cervical tissue locations via lymphatic channels.

4. Discussion

Many reports show that talc-containing powder, when applied to the perineum, has clear potential for migration to other pelvic organs and sites. For example, Cramer et al. [23], used SEM/EDX to show talc in several pelvic lymph nodes from a patient with ovarian carcinoma and a

history of perineal talc use. They reported that the nodal talc particles ranged in size from 5 to 10 μm, although they did not give more specific details on particle dimensions. A subsequent study [21] used tissue digestion and quantitative SEM/EDX to show that talc in pelvic lymph nodes statistically correlated with known talc use in patients with ovarian carcinoma, when contamination factors were properly controlled for. In another report, McDonald et al. [24] used SEM/EDX to demonstrate the presence of talc in a variety of pelvic sites (ovaries, lymph nodes, cervix, and/or uterus) in a series of five talc-exposed patients with ovarian carcinoma. The majority of talc particles identified in that study were small and roughly isodiametric rather than fibrous, with overall dimensions 1–10 μm, consistent with the study of Cramer et al. [23], and consistent with the most common size class found in the analysis of the TCBP and talc particles in ovarian carcinoma resections in the current study. Fibrous talc particles were found by McDonald et al. [21,24] (18 of 502 total talc particles, 4%), and they had an aspect ratio of approximately 3–6, with none reaching or exceeding 10. This is generally consistent with the dimensions and proportions of the large-aspect-ratio particles found and analyzed in the current study.

In this study, we used 11 randomly selected patients to achieve 200 talc particles within pelvic tissues resected at surgery for detailed study of particle size and shape. Our experience in analysis of particles and fibers in pelvic tissue was recently presented at a recorded, public US Food and Drug Administration meeting in February 2020 [25]. At that time, we reported that 196 ovarian cancer cases (with a history of talc use) had been studied by polarized light microscopy and birefringent particles were found in the pelvic tissues of 180 (92%) of those patients. Of those cases, 91 had been studied by SEM/EDX and 82 (90%) were found to have talc particles. The cases used in the study reported here ranged from 3 to 68 talc particles in planes of tissue ~2 μm in depth. In all our cases studied, most were under 100 particles per case, but we do have one case with over 500 and one with over 1000 talc particles per case. Therefore, the cases selected for this study were very representative of our experience using this morphological approach.

Gilbert et al. [26] studied two commercially available talc preparations used in pleurodesis and measured a median particle diameter of 26.57 μm (range 0.399 to 100.237 μm) in one preparation and 24.49 μm diameter (0.224 to 100.237 μm) in another. Our results, collected from 400 randomly found talc particles in TCBP, showed an average length of 19.29 μm $\pm$ 20.73 s.d. (range of 1 to 65 μm), which are consistent with these previously published results. It should be noted, though, that our samples represented talc sold over the counter, and intended for external bodily hygiene use, not for iatrogenic use in an interior anatomic compartment. Furthermore, talc particles used for pleurodesis are by design large particles because it is known that particles <5 μm in greatest dimension can enter the pulmonary lymphatics and may cause inflammatory pulmonary disease and respiratory failure [27–30].

Talc, when applied to the perineum, can gain access to other pelvic organs and sites either through retrograde ascension through the female genital tract, thereby reaching uterine tubes and/or ovaries [10–12,31,32], or through entry into small submucosal lymphatics. Entry into lymphatics could presumably be accessed through superficial erosions that occur in the epithelium of the lower female genital tract [24]. Talc could then migrate to many potential locations via lymphatics [13–15], including some (e.g., ovary) that could also be accessed via the retrograde ascension route. However, pelvic lymph nodes are a commonly expected and observed anatomic end site for lymphatic migration [21,23,24]. Talc may also lodge in the subserosal soft tissue of the uterus and uterine tubes; the source, at least in part, is presumed to be lymphatic migration, since the distribution of talc in such locations is often perivascular [24].

There were many similarities in talc particle dimensions between the consumer TCBP samples and the talc particles in the 11 ovarian carcinoma patients, suggesting that one is a plausible source for the other. Most particles in the two groups are small and isodiametric (Tables 1 and 2). Roughly similar proportions of talc particles in the two groups were of the small/isodiametric category and the longer aspect ratio/smaller size category. The size ranges in Table 2 were also similar to those presented in the aforementioned tissue-based study of McDonald et al. [24]. The vast majority of particles in the consumer preparations were talc, as expected. Within the patient samples, a large proportion of particles were talc in sites such as lymph nodes and subserosal lymphatics. However, when all patient tissues and sites are considered in aggregate, a substantial number of birefringent particles may be other categories, particularly silicates. These may be absorbed into the perineum through general living practices, in a manner similar to talc, and then also be absorbed or disseminate locally into other tissues. For example, a substantial number of silicates along with talc were demonstrated by SEM/EDX in the tissue-based study of five talc-exposed ovarian carcinoma patients by McDonald et al. [24]. The analysis of the commercially available TCBP samples indicates that contaminants of these commercial samples may be an important source of exogenous nontalc silicate particles [33].

Talc is clearly capable of inducing inflammation. This has been demonstrated by the stimulation of macrophage activity in animal models [34,35] the presence of variable macrophage, giant cell, or lymphocytic infiltrates in talc-exposed human tissues depending on the patient and anatomic site [24,36] and the increase in proinflammatory markers in the setting of talc exposure in in vitro models [9,37] and the tissue response to in vivo pleurodesis [36]. Talc is known to have a slow dissolution rate in tissue, on the order of years [38], thereby indicating that talc may be capable of stimulating inflammation and then remaining as a source of inflammation for many years following exposure. Through its interaction with macrophages and ovarian carcinoma cells in vitro, talc is capable of generating reactive oxygen species, which are known to promote the occurrence of mutations which stimulate and enhance carcinogenesis [9]. The development of malignancy occurs gradually over time and in multiple molecular stages, with the persistence of talc in tissue again being a key factor that would make carcinogenesis more likely from a biochemical standpoint.

It is important to note that talc particles (corresponding to birefringent material seen by PLM) that were found in the surgically resected tissues from ovarian carcinoma patients were typically in benign rather than malignant tissue, e.g., see the collections of talc particles in the macrophages in resected lymph nodes (Fig. 2). Often such particles were found within inflamed stromal or fibroblastic tissue in ovaries, with tumor nearby. Talc does not need to be found within the actual tumor tissue to support a causal link, the presumption being that it accumulates in benign ovarian or other female genital tract tissue some time before tumor develops.

Lymphatics present in submucosal and/or peripheral locations, such as the ones presumably accessed by perineal talc, are typically tens of microns in diameter [15], well within the range of the microbes, cells, and particulate matter that the lymphatic system typically transports, including the talc studied in the TCBP preparations. Thus, the size range of the talc particles demonstrated in the consult patients in this study (average 5.78 μm, $\pm$ 4.02 s.d.) is consistent with a lymphatic route of transport, though by itself does not prove it. Lymphatic channels are probably also more efficient at transporting isodiametric particles, which were the most commonly encountered category of talc in this study. High-aspect-ratio talc fibers could theoretically enter a lymphatic channel in a longitudinal fashion, but they would have a greater chance to become trapped early in the transport process by impaction or interception at convolutions or branch points in the lymphatic pathway.

In conclusion, our study lends support to the contention, also evidenced by other literature reports, that externally-applied talc can migrate from the perineum to other pelvic sites, and that commercially available talc powder is consistent in particle size and dimensions with the typical talc particles, found by SEM/EDX, that are lodged in surgically resected pelvic tissues of ovarian carcinoma patients.

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Conflict of interest statement

KEJ worked as a paid expert medical witness for the Beasley Allen Law Firm in 2018 and 2019. JJG has served as a consultant and provided expert testimony in talc and other environmental litigation. AP, SM, and YF report no conflicts of interest.

Authors contributions

KEJ conceived, planned, did most of the writing, assisted on SEM/EDX for TCBP, assembled data sets, and summarized the project. AP did the SEM/EDX studies for TCBP. JJG, YF, and SM wrote most of the discussion and did microscopy for ovarian carcinoma resections. All authors contributed to manuscript writing and editing.

Declaratiion of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2020.09.028.

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